PROGRAM AND ABSTRACTS OF PAPERS CITRUS RESEARCH CONFERENCE

FRUIT AND VEGETABLE
CHEMISTRY LABORATORY
263 SOUTH CHESTER AVENUE
PASADENA, CALIFORNIA 91106

December 2, 1970

Western Utilization Research and Development Division
Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in Southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Utilization Research and Development Divisions of the Agricultural Research Service, U. S. Department of Agriculture. The following are participating in this year's conference.

- Western Utilization Research and Development Division:
  - Western Regional Research Laboratory (Division headquarters), 800 Buchanan Street, Albany, Calif. 94710
  - Fruit and Vegetable Chemistry Laboratory, 263 South Chester Avenue, Pasadena, Calif. 91106

- Southern Utilization Research and Development Division:
  - U. S. Fruit and Vegetable Products Laboratory, 600 Avenue S, N.W., Winter Haven, Florida 33882
  - U.S. Food Crops Utilization Research Laboratory P.O. Box 388, Weslaco, Texas 78596

P R O G R A M

CITRUS RESEARCH CONFERENCE

Wednesday, December 2, 1970

CHEMICAL REGULATION OF COLOR FORMATION IN CITRUS FRUITS

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Recently we reported (Science 168: 1589, 1970) on the accumulation of lycopene induced by 2-(4-chlorophenylthio) triethylamine hydrochloride (CPTA) in a wide array of carotenogenic tissues, including a number of citrus fruits. Where response was noted, lycopene accumulated as the principal pigment regardless of the pattern of carotenoid distribution on the untreated samples. Preharvest and postharvest treatments of citrus fruits were equally effective.

When Marsh grapefruit, Washington navel orange, Valencia orange, Eureka lemon, Satsuma mandarin, and Sinton citrangequat were treated with CPTA, lycopene accumulated as the principal pigment in the peel. The compound caused the accumulation of higher concentration of lycopene in the peel of Redblush grapefruit. Lycopene is normally present as the predominant pigment only in the red-pigmented grapefruit and pumelo varieties. Otherwise, except as a minor constituent in immature Marsh grapefruit, two orange cultivars and tangerine, lycopene has not previously been reported to accumulate in citrus fruit.

When the fruits were immersed for 30 seconds in solution containing 5000 p.p.m. of CPTA, accumulation of lycopene occurred within a few days in small irregularly shaped patches of tissues. When fruits were held for several weeks or longer subsequent to treatment, lycopene accumulated throughout the flavedo and also to some extent in the albedo. The response observed in the latter tissue was dependent on the degree of penetration of the compound. In the Sinton citrangequat fruit which has an extremely thin peel, lycopene accumulation was noted in the endocarp, indicating the effectiveness of the compound in this tissue. The oxy analog of CPTA also caused citrus peel tissue to accumulate lycopene.

It is apparent that CPTA stimulates the lycopene pathway. Its mode of action in carotenoid biosynthesis, whether at the enzyme or gene level, and its effect on other terpenoid constituents are currently under investigation.

RECENT PROGRESS IN SWEETENER RESEARCH

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The banning of cyclamate as a food additive last year and more recently as a drug has focused attention on the fact that there is an increasing, worldwide demand for a non-toxic, non-nutritive sweetener and flavor enhancer. The applications of this type of compound in foods, beverages, confections, pharmaceuticals and related preparations are well known. Sweetness is obviously one of the most useful and sought after taste characteristics, but one which is extraordinarily difficult to achieve in a satisfactory manner in the numerous instances where it is neither possible nor desirable to provide it from straight carbohydrate sources.

Among the compounds that are strong contenders for replacing cyclamate are the dihydrochalcones prepared from citrus flavonoids. The three most promising of these are naringin dihydrochalcone (prepared from naringin), neohesperidin dihydrochalcone (from neohesperidin or from the conversion of naringin) and hesperetin dihydrochalcone glucoside (HDG) (from hesperidin). During the past year we have explored the effects produced by modifying substituents in the B-ring and sugar portion of the dihydrochalcones. The results of this work will be reviewed.

The decision to carry out two-year feeding tests at the Western Regional Research Laboratory on naringin and neohesperidin dihydrochalcones has necessitated the synthesis of large quantities of these compounds. Since the large scale preparation of neohesperidin dihydrochalcone requires a number of steps starting from naringin, the reactions must be monitored closely to assure high purity of the final product. Consequently, several analytical methods have been devised to detect the various possible impurities in neohesperidin dihydrochalcone prepared by this process. These include paper electrophoretic and thin layer procedures. A reagent has been discovered that appears to be highly specific and sensitive for detecting dihydrochalcones.

A brief summary will be given of related developments in the field of non-nutritive sweeteners.

OPTICAL ROTATION OF CITRUS FLAVANONES AND ITS RELATIONSHIP TO TASTE AND BIOSYNTHESIS

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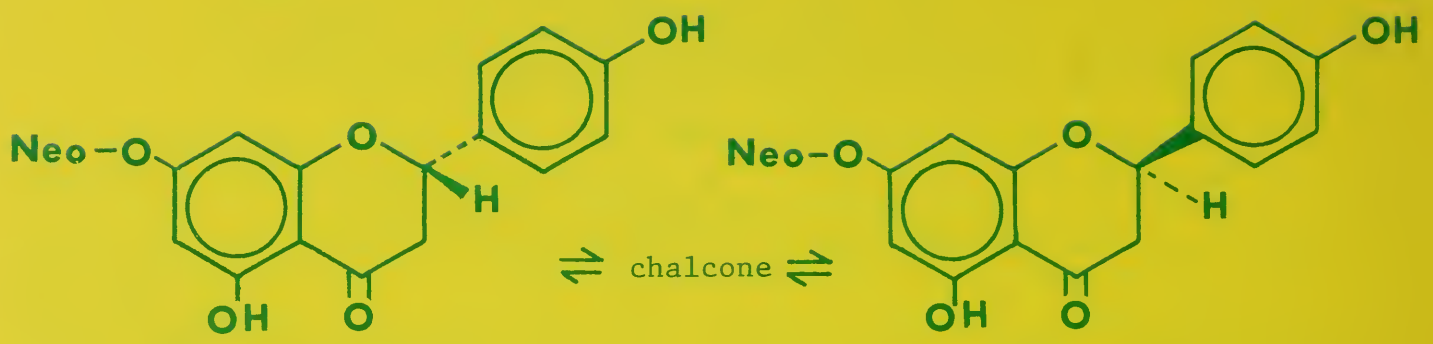
Asymmetric carbon atoms present in flavanone glycosides are located in two portions of the molecule, the carbohydrate moiety and at C_2 of the aglycone. The asymmetric centers give rise to optical rotation at the sodium D line (Shimokoriyama, J. Amer. Chem. Soc. 79: 4199, 1957) and to optical rotatory dispersion (ORD) in the ultraviolet region at 225-360 nm. (Gaffield and Waiss, Chem. Commun. 29, 1968). The latter rotations occur as optically active absorption bands or Cotton effects. Circular dichroism (CD), which is the differential absorption of right- and left-handed circularly polarized light, is preferred over ORD measurements owing to less complication of the data by overlapping effects. Thus, CD has been used to study an extensive series of flavanones, 3-hydroxyflavanones and their glycosides in order to relate their absolute configuration at C_2 to the sign of various Cotton effects (Gaffield, Tetrahedron 26: 4093, 1970). Flavanones of 2 S configuration, having equatorial 2-aryl substituents, exhibit a positive Cotton effect due to the $n \rightarrow \pi^*$ transition ($\sim$ 330 nm.) and a negative Cotton effect in the $\pi \rightarrow \pi^*$ region ($\sim$ 280-290 nm.). Flavanone glycosides possessing chiral aglycones show Cotton effects quite similar to their optically active aglycones while flavanone glycosides having racemic aglycones show only weak Cotton effects at 250-350 nm. The $\pi \rightarrow \pi^*$ Cotton effect is most suitable for determining aglycone chirality in flavanone glycosides. Therefore, CD may be used to determine or monitor the stereochemistry at C_2 of flavanone glycosides without interference from the sugars present in the molecule.

We have studied the aglycone chirality of naringin, the main bitter principle in grapefruit, as a function of grapefruit maturity. The 2 S:2 R ratio of isomers varies as the fruit develops. The amount of 2 S isomer is 85-92 percent in young fruit while it is only 55-60 percent in mature grapefruit. These findings are of interest as they concern effect of stereochemistry upon taste properties and for possible biosynthetic implications.

Taste differences among stereoisomers such as amino acids and peptides are well known. The diastereoisomeric 2 S and 2 R naringens have different molecular shapes. Different taste properties could account for the diminishing bitterness as the fruit reaches maturity. However,



2 S Naringin 2 R Naringin

preliminary tasting indicates that the R isomer is as bitter as the S compound. Other preliminary tasting studies have found 2 S and 2 R neohesperidin to be equally bitter. Thus the stereochemistry at C_2 in the aglycone of flavanone glycosides does not appear to have a major effect upon the taste properties of these compounds although we have not yet studied the optically pure 2 R isomers.

Grisebach ("Biosynthetic Patterns in Microorganisms and Higher Plants," John Wiley, 1967) has shown that 2 S-flavanones are stereo-specifically incorporated into quercetin and cyanidin. Thus 2 S-flavanones are the natural intermediates for biosynthetic incorporation. The change in configuration at C_2 of naringin may be related to this process. One hypothesis to accommodate our experimental findings is as follows. 2 S-Naringin is formed originally in the new fruit by a chalcone-flavanone isomerase (CFI). As growth proceeds some of the 2 S-naringin is utilized biosynthetically and some is converted nonenzymatically back into the chalcone. Such a non-enzymatic process is plausible since naringin is known to form the chalcone with extreme ease (Horowitz and Jurd, J. Org. Chem. 26: 2446, 1961). If the CFI is inhibited as the fruit ripens then the naringin reformed from the chalcone will be racemic.

FLAVANONE CONTENT OF WHOLE GRAPEFRUIT AND JUICE AS INFLUENCED BY FRUIT DEVELOPMENT

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(to be presented by Steven Nagy)

The purpose of the research reported here is to establish to what extent the leaves of citrus influence the nature of flavanone components accumulated in the fruit and to determine if the nonbitter naringenin-7-rutinoside accumulates at the expense of the bitter naringin as fruit develops and matures.

Prior workers have established that grapefruit accumulates the majority of its principal flavanone components during the very early stages of fruit development, and that little synthesis and accumulation occurs beyond that time.

Redblush grapefruit of approximately 3 cm. in diameter was grafted onto potted trees of five different citrus taxa: Poncirus trifoliata, Citrus aurantium, C. aurantifolia, C. macrophylla, and Troyer citrange. After six months of development and maturation on these foster-mother trees, the fruit was harvested and the flavanones isolated from the juice. The two naringenin rhamnoglucosides were quantitated by the TLC-fluorometric procedure. Although the majority of the flavanones were already accumulated in the grapefruit at the time they were removed from the parent tree for grafting, it appears that there are minor alterations of the ratio of neohesperidose to rutinose glycosides of naringenin in the juice. The direction of the shift in this ratio was consistently in that direction which approached the ratio characteristic of the fruit of the foster-mother tree.

Budwood of Redblush grapefruit was grafted onto a mature sour orange seedling. After one year of normal growth, all leaves were removed from the budwood shoot at the time of blossoming. Fruit developed from these blossoms in the absence of any grapefruit leaves. After 4 and 8 weeks of development, the fruit was harvested, and the six major flavanones were quantitated. Fruit of the same age from the foster-mother tree (sour orange) and from a commercial grapefruit tree (on sour orange rootstock) was also analyzed.

Although the grapefruit from the budwood on the foster-mother tree developed from blossom to over 30 grams during the period when most flavanones are synthesized and accumulated, its flavanone composition

closely approximates that of grapefruit grown on the commercial grapefruit tree and does not show any tendency toward an increase in hesperetin glycosides nor in neohesperidosides as is characteristic of the fruit from the sour orange seedling.

When one examines the content of individual flavanones relative to total flavanones in grapefruit from ovary stage to market maturity, it is apparent that no significant changes in relative composition are occurring. This relative constancy of flavanone aglycone and glycoside during development and maturation suggests that little, if any, interconversion occurs.

THE APPLICATION OF AMINO ACID COMPOSITION TO THE CHARACTERIZATION OF CITRUS JUICES*

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The total amino acid composition of lemon juice has proved to be quite valuable in determining the authenticity of a juice sample. Preliminary investigations in this laboratory and other published reports indicate that the total amino acids will be useful in determining orange juice authenticity or juice content. However, total amino acid values have the possible limitation that they could be adjusted with one or more amino acids.

The amino acid pattern on paper chromatography or thin-layer chromatography could be used to qualitatively detect gross adulterations. There are, nevertheless, times when a more precise measure of the individual amino acids is needed. Ion-exchange column chromatography was chosen as the method to be used for studying the individual amino acids of citrus juices.

An amino acid analyzer was designed and built from commercially available, inexpensive parts. The design and operation will be briefly discussed.

The neutral and acidic amino acids were resolved on a 0.9 x 50-cm. column of Aminex Q-1505 (Bio-Rad) by using lithium citrate buffers with a pH gradient. The basic amino acids were resolved on a 0.9 x 15-cm. column of the same resin with a sodium citrate buffer.

The major amino acids in orange juice were proline, arginine, γ-amino butyric acid, asparagine and aspartic acid. Lemon juice had relatively large amounts of proline, asparagine, aspartic acid, serine, alanine and glutamic acid.

Some of the amino acids in orange and lemon juices seem to be present in fairly constant amounts while others appear to be a function of variety, growing area or climate. For example, there is more asparagine than aspartic acid in coastal California lemons while the reverse is the case in Arizona and Florida lemons. The percentage of proline is also higher in coastal California lemons than in either Arizona or Florida fruit. The ratios and percentages of some of the amino acids will be discussed in relation to chemically characterizing citrus juices.

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*This research was supported in part by the Lemon Products Technical Committee, Los Angeles, California.

INVESTIGATION OF THE PARTICULATE MATTER OF LEMON JUICE*

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The objective of work now in progress is to survey a variety of methods that may aid in understanding the nature of lemon juice particles. Implementation has been based principally upon centrifugation and particle size estimation via electronic counting. Centrifugation was at first limited to a maximum g-force of about 35,000; 10-minute centrifugation at this comparatively low g-max was adequate, however, to produce pellets that could be readily separated from supernates. By dissolving KBr in juice, decantate densities ranging from 1.04 to 1.23 g./ml. were obtained. While particles were found throughout this range of densities the greatest contribution to dry pellet mass was accounted for by particle densities between 1.1 and 1.2 g./ml.

Availability of a preparative ultracentrifuge has made it possible to operate a zonal rotor with g-forces as high as 171,800. In the first use of this rotor a 10-ml. sample of freshly prepared lemon juice was mixed with 40 ml. of 0.125 M NaCl. This 50-ml. sample was injected over a linear gradient ranging from 10 to 60 percent sucrose. After providing a 50-ml. overlay of 0.125 M NaCl the rotor was brought to full speed (g-max 171,800) and held there for 4 hours. The content of the rotor was collected as 39 fractions. These fractions are being examined with a Model A Coulter counter; it is hoped that profiles of particle distribution can be prepared from the resulting data. If circumstances are favorable it will eventually be possible to fractionate lemon juice particulate matter in a useful way with the zonal rotor.

Nearly all of the results obtained are based upon hand-extracted juice. Batches of several hundred milliliter were first strained through a single layer of cheesecloth, then passed through an EC glass frit and finally through a C frit. For the very small samples needed for particle counting or zonal rotor work the fresh juice was passed immediately through the C porosity fritted glass filter. Although this juice is not typical of industrial products it appears to be more amenable to experimentation than juices that contain larger particles. The turbidity of the experimental juice does not differ markedly from that of whole juice, and it may prove useful to regard it (or a suitable derivative) as a precursor of commonly encountered varieties of juice. During the early centrifugation experiments a pronounced and regular dependence of dry pellet mass upon juice age was

*This research was supported in part by the Lemon Products Technical Committee, Los Angeles, California.

observed. This dependence strongly suggests the occurrence of mass transport between juice particles and serum. It is hoped that it will be possible in future work to clarify the nature of these effects and to judge their significance in commercial processing. Interrelation with the effects of pasteurization and juice concentration is clearly of interest and is equally subject to scrutiny.

CHEMICAL STIMULATION OF LIMONOID METABOLISM: AN APPROACH TO PRODUCTION OF NONBITTER NAVEL ORANGE JUICE*

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In recent years we have been studying the biochemistry of limonin in an effort to find a solution to the problem of delayed bitterness in citrus juices. Last year we reported the development of a highly specific and sensitive quantitative thin layer chromatography method for determining the limonin and potential limonin contents of citrus fruit tissues and juices (J. Agr. Food Chem. 18: 250, March-April 1970). Since its development the method has played an important role in our studies of the limonin bitterness problem.

It has been observed over the years that navel oranges harvested up to several months after becoming ripe (early- to mid-season fruit) produce juice that undergoes delayed bitterness whereas fruit harvested several months later (late-season fruit) produces nonbitter juice. Subsequently, we showed that navel oranges that produce bitter juice contain limonoate A-ring lactone (the nonbitter precursor of limonin) in the endocarp tissues while those that produce nonbitter juice do not contain that compound. Thus, as navel oranges approach late-season maturity, limonoate A-ring lactone gradually disappears from the fruit tissue. This observation is important because it shows that the limonoate A-ring lactone content of the fruit is in a dynamic state, and that native enzymes are capable of gradually metabolizing it to nonbitter products. Considerable time, however, is required after commercial maturity is reached for the enzyme system to metabolize sufficient limonoate A-ring lactone to result in nonbitter juice. Thus, while it is clear that the fruit possesses an enzyme system which metabolizes limonoate A-ring lactone, the system is not sufficiently active for commercial purposes. However, if a means could be found to induce greater activity of this enzyme system, limonoid metabolism would be accelerated and nonbitter juice could be obtained.

On the basis of these biochemical facts, we have been searching for compounds which promote the metabolism of limonoate A-ring lactone by the intact fruit. With such a compound the fruit destined for processing into juice could be treated at the packing house immediately after being separated from the fruit going to the fresh market. Treatment at this point would take advantage of the transit time from packing house to processing plant to allow metabolism of the limonoate A-ring lactone. Although this

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*This research was supported in part by the Citrus Advisory Board, Los Angeles, California.

short time might not be sufficient to yield juice completely free of limonin bitterness, the level of bitterness might be reduced significantly. In addition, the fruit could be held longer at the packing house after treatment or at the processing plant in outdoor bins to allow longer time for debittering. A particularly useful chemical agent would be one that accelerates limonoate A-ring lactone disappearance substantially in several days.

We have recently found a compound, 2-chloroethylphosphonic acid, that looks promising in this regard. Although its commercial potential is difficult to judge at this early date, results obtained so far are very encouraging. (2-Chloroethylphosphonic acid has not been cleared by Food and Drug Administration for commercial use on citrus for this purpose.)

We find that when an aqueous solution of 2-chloroethylphosphonic acid is applied to the surface of detached citrus fruits there is an appreciable acceleration in the disappearance of limonoate A-ring lactone from both the peel and the juice portions of the fruit. The net result of the treatment is lower limonin levels in the juice. For example, the limonin content of juice from navel oranges and lemons treated with 2-chloroethylphosphonic acid and held at 70°F. dropped 20 to 40 percent in five days. Reductions in the limonoid content of the peel were somewhat larger. In these studies extreme care was taken to obtain uniform lots of fruit and to prepare juice and peel samples in a consistent manner. Variations of limonin levels in peel and juice from replicate samples of fruit were less than 5 percent.

Further research is in progress on the use of 2-chloroethylphosphonic acid to reduce the limonin bitterness of citrus juices. Also, its effect on other juice constituents and on organoleptic quality will be evaluated. Other research in progress at our Laboratory on limonin metabolism should lead to important information about the enzyme system and products of the chemically accelerated metabolism of limonoate A-ring lactone.

METABOLISM OF LIMONIN BY MICROORGANISMS: THE CONVERSION TO NONBITTER COMPOUNDS

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In dealing with the problem of limonin bitterness of citrus juices, several approaches are possible. When specifically considering fruit which already contains sufficient limonoate A-ring lactone to produce bitter juice, two general lines of attack are apparent. The first is that of preprocessing treatments dealing with the intact fruit before the juice is extracted. Research along these lines is underway at our Laboratory and is reported elsewhere in this publication (see Maier and Brewster). The second approach, processing treatments, deals with the juice itself during extraction and processing. Considerable research has been done in this area over the years. Of the various methods that have been demonstrated, the most practical seems to be that of Higby (1941). He reported that bitterness of navel orange juice could be minimized by avoiding tissue maceration during juice extraction and by immediately separating the coarser tissue fraction from the extracted juice. Although helpful in reducing limonin levels, even with extreme care juice bitterness cannot be entirely prevented by this method, and juice yields are reduced. Methods which depend on physical removal of limonin from the juice have not thus far proved to be commercially feasible.

We are presently exploring another method of eliminating juice bitterness, namely, enzymic conversion of limonin into nonbitter products. In this regard, a search is underway for enzymes capable of catalyzing the debittering of limonin in citrus juices.

With this objective in mind we have made a general survey of microorganisms and found several species of bacteria that grow well on a medium containing limonin as a single carbon source. During microbial growth the limonin concentration of the medium decreased and several new limonoid compounds appeared, thereby confirming the fact that the organisms were metabolizing limonin. Two metabolic products, which were Ehrlich's reagent positive, were isolated and characterized by thin-layer chromatography and nuclear magnetic resonance spectroscopy. One of the metabolites was identified as deoxylimonin and the other one as deoxylimonic acid. The fact that both of these limonoids are nonbitter is of important practical significance. Microbial metabolism of limonin to these products demonstrates the existence of enzymes capable of catalyzing these

debittering reactions. In addition, the microorganisms provide a potential source of the enzymes.

Further investigations on characterization of other metabolites, isolation and characterization of the organisms, and isolation of enzymes involved in the metabolism of limonin are in progress.

STUDIES ON THE BIOSYNTHESIS AND METABOLISM OF LIMONIN

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A rational approach to the control of limonin bitterness in processed citrus juice requires an understanding of the biochemical mechanisms involved in the synthesis and metabolism of limonoids. The structure of the major citrus limonoids have been established in recent years, and some of them can be fitted into a plausible biosynthetic pathway leading to limonin. It was therefore of interest to look for minor citrus limonoids which could possibly fill in gaps in the proposed biosynthetic sequence and/or provide evidence about the metabolism of limonin. In particular, acidic limonoids seemed worth investigating since methods used previously for isolating limonoids would have missed them.

A grapefruit seed extract, from which most of the limonin had been removed by crystallization, was separated into neutral and acidic fractions. Thin-layer chromatography (TLC) of the acidic fraction showed the presence of five compounds giving the characteristic limonoid color reaction with Ehrlich's reagent. Each of these was then isolated by chromatography and characterized. Two of them proved to be identical with synthetic samples of isoobacunoic acid and epiisoobacunoic acid, neither of which was previously known to occur in nature.

The other three compounds were new limonoids. Two of them have now been shown to be the open A-ring derivatives of nomilin and deacetylnomilin, and they have been named nomilinic acid and deacetylnomilinic acid, respectively. Unlike the case of limonin, these compounds are relatively stable in the hydroxy acid form and are only lactonized with difficulty.

The other new acidic compound is apparently a unique type of limonoid, having C-19 linked to C-4 through an oxide bridge. It could be derived from deacetylnomilinic acid by oxidation of the 19-methyl to hydroxymethyl and subsequent ring closure. An alternative possibility could be a transannular reaction, in which the 19-methyl group is attacked by the C-4 oxygen function. Molecular models indicate that this simultaneous oxidation and ring closure is stereochemically feasible.

The neutral fraction of the grapefruit seed extract was examined by TLC. It contained, in addition to large amounts of the known limonoids,

two minor Ehrlich-positive components slightly more polar than deacetylnomilin. One of these was found to be identical with ichangin, which Dreyer had previously isolated from Ichang lemons. The second compound was identified as deoxylimonol, a new limonoid which could be a metabolite of limonin.

Several of the compounds isolated during this work are possible precursors of limonin, and the biosynthetic implications of these findings will be discussed. However, attempts to detect metabolites of limonin were largely unsuccessful. A different approach to the study of limonin metabolism has therefore been started. A radioactive derivative of limonin was prepared and administered to ripening fruits, and its metabolism was followed by isolating the radioactive products. The initial results of this approach will be discussed.

CONVERSION OF (+)-LIMONENE TO USEFUL SESQUITERPENES

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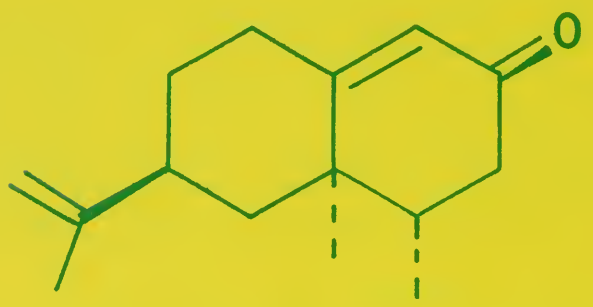
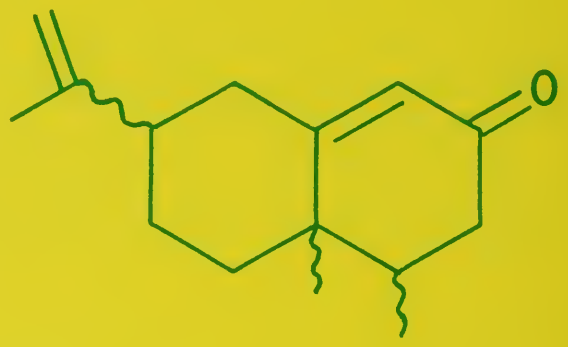
(+)-Limonene comprises about 95 percent of orange oil and is, therefore, an inexpensive compound available in amounts perhaps as large as 50 million pounds per year. The conversion of (+)-limonene to useful sesquiterpenes by way of an easy three-step synthesis will be discussed. The products differ from nootkatone (the flavor principle of grapefruit) only in the position of the isopropenyl group and the relative stereochemistry of the isopropenyl and the methyl groups.



Nootkatone



Reaction products

The components of the reaction mixture were separated by preparative gas-liquid chromatography. The structures of the major isomers were determined from their spectroscopic properties. Sensory evaluations, as well as possible commercial applications of the isolated sesquiterpenes, will be presented.

THE ROLE OF LIPIDS IN OFF-FLAVOR DEVELOPMENT OF COMMERCIALLY PREPARED ORANGE JUICE

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Commercially processed citrus juice is highly susceptible to off-flavor and off-odor development when stored at adverse temperatures and for prolonged storage periods. Previous work from this Laboratory showed that the suspended matter of citrus, which includes the lipid fraction, was the principal contributor to off-flavor in aged orange juice.

In this study, the effects of storage temperatures and length of storage on the neutral lipid and polar lipid composition of chilled orange juice were sought. Over a 16-month storage period the neutral lipid composition of 40°F. stored juice increased 11 percent while juice stored at 85°F. showed an increase of 63 percent. Thin-layer chromatographic densitometry showed this increase to be due to enhanced formation of free fatty acid. Inspection of two major neutral lipids, viz., steryl ester and triglyceride, showed minimum breakdown and, therefore, could not possibly account for the large concentration gain in the free fatty acid fraction. The lipids that showed enhanced enzymic hydrolysis at elevated storage temperatures have been traced to the polar lipid fraction. After 16 months' storage at 85°F., the phospholipid concentration, as measured by phospholipid phosphorus, decreased to 30.4 percent of its original value. The phospholipids most susceptible to breakdown at elevated storage temperatures were phosphatidyl-choline, -ethanolamine, -inositol and -serine. From the breakdown of phospholipids, it was possible to account for a considerable percentage of the free fatty acid increase manifest in the neutral lipid fraction, viz. 94.6 percent.

From our studies it appeared, a priori, that neutral lipase activity was relatively weak while phospholipase activity was quite pronounced. The production at elevated storage temperatures of large quantities of unsaturated fatty acids predisposes orange juice to off-odor and off-flavor development. High carbon number fatty acids, as found in orange juice, contribute very little to flavor; however, they are important as precursors to many volatile off-flavor compounds. Studies are currently being conduct on identifying and following the production of off-flavor compounds during storage.

THE ANTIMICROBIAL ACTIVITY OF CITRAL

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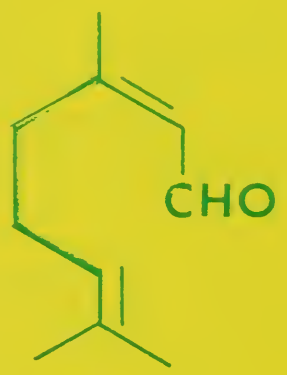
A search for naturally occurring antimicrobial substances has led to an examination of citral. Several workers have reported that citral is active against a wide variety of bacteria, yeasts, and molds at various concentrations. In most instances, commercial citral was used, and the purity of the material was not given. Our work initially confirmed their findings in that commercial citral was active against a representative sample of 10 bacteria, 6 yeasts, and 7 molds. Citral which was purified by forming the bisulfite addition compound, recrystallization, regeneration with acid, and distillation still showed the same activity. The activity is slightly less than commercially used Dowicide. However, when citral was separated into its two components, neral (1) and geranial (2), by preparative gas-liquid chromatography (GLC) we found that the separated isomers had much less activity. Also, when the two pure geometric isomers were mixed in a 50:50 ratio, activity was still absent. It is thus apparent that much of the activity of citral is due to a minor component which is carried through the usual purification process. Also, the activity of the impurity(ies) must be substantial because the "pure" citral was approximately 98 percent pure (by GLC).



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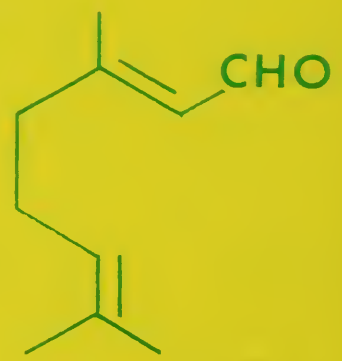
Neral



2
Geranial

LIST OF PUBLICATIONS AND PATENTS*

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